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Studies of the Expression of the Cytochrome P450IA, P450IIB, and P450IIC Gene Family in Extrahepatic and Hepatic Tissues

by Thomas Friedberg,* Peter Siegert,* Markus A. Grassow,* Barbara Bartlomowicz,* and Franz Oesch*

We have studied the expression of three P-450 gene subfamilies in hepatic and extrahepatic tissues using the sensitive RNAse A protection assay. Members of the P450IA subfamily, which encodes the major methylcholanthrene-inducible cytochromes P-450, were found to be not expressed in extrahepatic tissues of untreated animals, raising the question whether these P-450 play a role in the metabolism of carcinogens in unexposed individuals. In contrast, members of the P450IIB family, some of which encode the major phenobarbital-inducible cytochromes P-450, were found to be expressed in some extrahepatic tissues of untreated rats and here most notably in the lung and in sebaceous glands. Members of the P450IIC family, which encode some constitutively expressed cytochromes P-450, were found to be expressed exclusively in the liver.

Introduction

Cytochromes P-450 are a large family of monooxygenases that play an important role in the metabolism of a wide variety of endogenous and exogenous compounds including steroids and polycyclic hydrocarbons (*1*). Recently several P-450 cDNAs and genomic clones have been isolated and sequenced, making it possible to order the various cytochrome P-450 genes in families and subfamilies on the basis of sequence comparisons (*2,3*).

Cytochromes P-450 have been mainly studied in the liver, which is certainly not the target organ for most chemical carcinogens. This approach has been chosen because the liver consists mainly of a single cell type, the hepatocyte, and because this organ contains a considerable amount of several cytochromes P-450, some of which can be induced by xenobiotics. Only very few studies have been concerned with cytochromes P-450 in extrahepatic tissues (*4–6*). In these studies antibodies against purified cytochrome P-450 isozymes have been used.

Only recently specific oligomer probes for cytochrome P-450 genes have been used to study their expression in several tissues (*7*). However, experiments involving oligonucleotides will only show whether a given probe is able to recognize an mRNA, but will usually not reveal the extent of recognition. Thus, these methods are not useful for the identification of a previously unknown member of a given cytochrome P-450 gene family. On the other hand, Myers and Maniatis (*8*) have developed a method based on the RNAse A protection assay which will allow the detection of single point mutations in large stretches of the β-globine gene. In this assay a radiolabeled anti-sense RNA probe, which is generated *in vitro*, is hybridized to RNA isolated from a tissue. The resulting duplex molecules are treated with RNAse A, which is known to cut into imperfectly matched duplex molecules.

With this method we have studied the expression of the P450IA family, which encodes the major methylcholanthrene-inducible cytochromes P-450; the expression of the P450IIB family, which encodes the major phenobarbital-inducible cytochromes P-450; and the expression of the P450IIC family, which encodes some cytochromes P-450 that are constitutively expressed in several tissues.

Results

Tissue-Specific Expression of the P450IA Family

The P450IA1 (1957–2620) transcript (numbers in parentheses indicate the start and the end of the transcript

*Institute of Toxicology, Johannes Gutenberg University, D-6500 Mainz, Federal Republic of Germany.

Address reprint requests to T. Friedberg, Institute of Toxicology, Johannes Gutenberg University, D-6500 Mainz, Federal Republic of Germany.

numbered from the initiation codon) was used for the RNAse A protection assay with RNA isolated (*9*) from several tissues (Fig. 1). The *in vitro* transcribed anti-sense RNA (Fig. 1, lane 14) consisted mainly of a full length transcript. RNA isolated from the liver of Aroclor 1254-treated animals but not that from untreated animals protected a large amount of the probe (Fig. 1, lanes 1 and 2 vs. lane 3). RNA from the lung and the kidney of Aroclor-treated animals protected considerably less RNA probe than did hepatic RNA from treated animals. Only a weak signal was obtained with RNA from the intestinal mucosa of Aroclor-treated animals (Fig. 1, lane 10). No signal was obtained with RNA isolated from the kidney and the intestinal mucosa of untreated animals nor with the RNA isolated from the testis of untreated and treated rats nor from the brain of male and female rats.

Tissue-Specific Expression of the P450IIB Family

The P450IIB1 (133–499) anti-sense RNA was used in the nuclease S1 and RNAse A analysis of RNA isolated from several tissues. Nuclease S1 protection assay of mRNA from untreated animals and of Aroclor 1254-treated animals (Fig. 2) yielded a protected fragment which had the same size as the fragment generated in the protection assay of the *in vitro* transcribed unlabeled P450IIB1 (133–499) sense transcript (Fig. 2). As expected, the amount of this fragment increased following Aroclor treatment. The RNAse A protection assay of mRNA isolated from Aroclor-treated animals yielded an intense signal for a fragment that had a slightly lower size than the fragment seen in the nuclease S1 protection assay (Fig. 2). A very small amount of this fragment was also seen in the RNAse protection assay of mRNA from control animals; however, this RNA yielded several additional fragments that were hardly seen in the analysis of mRNA isolated from Aroclor-treated animals (Fig. 2). This result clearly shows that the liver of untreated animals contained a P450IIB1-related mRNA that was suppressed by Aroclor treatment.

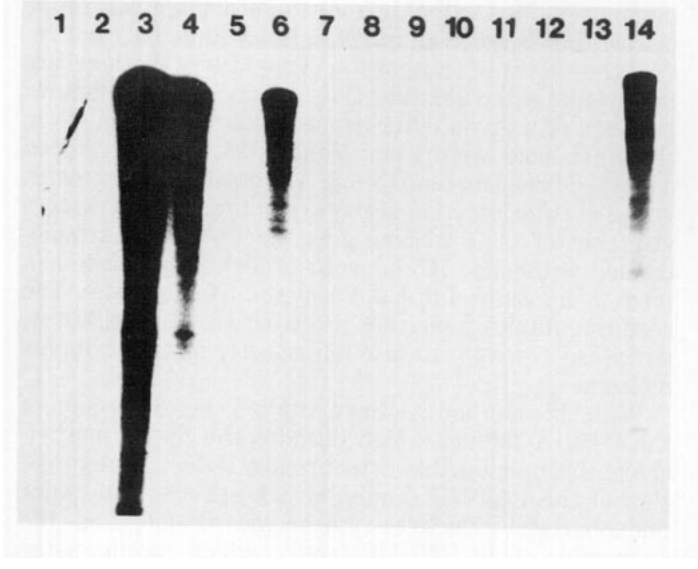

FIGURE 1. Detection of cytochrome P450IA RNA in various tissues. The P450IA1 transcript was used in the protection assay with RNA isolated from several tissues (lanes 1 and 2) liver of female and male rat, respectively; (lane 3) liver of treated rat; (lane 4) lung of treated rat; (lanes 5 and 6) kidney of untreated and treated rat, respectively; (lanes 7 and 8) testes of untreated and treated rat, respectively; (lanes 9 and 10) intestinal mucosa of untreated and treated rat, respectively; (lanes 11 and 12) brain of male and female rat, respectively; (lane 13) no RNA added to assay; (lane 14) probe.

mRNA isolated from the lung and the intestine of treated animals (Fig. 2) yielded the same RNAse A-protected fragment as the *in vitro* transcribed P450IIB1 (133–499) sense transcript. mRNA isolated from the testis, the brain, and the kidney did not give any distinct RNAse A- or nuclease S1-protected fragment. However, RNA isolated from the preputial gland yielded a large amount of several partially RNAse A-protected fragments that were distinct from the fragments obtained in the analysis of RNA isolated from control animals.

Tissue-Specific Expression of the P450IIC Family

Two probes were used to study the expression of the genes of the P450IIC family. One probe was complementary to the P450IIC6 cDNA, the other probe was complementary to the P450IIC7 cDNA. With the P450IIC7 (613–763) probe, a signal was only obtained with hepatic RNA from male and female rats, independent of whether or not they had been treated with Aroclor 1254 (data not shown).

With the P450IIC6 (556–763) probe, two protected fragments were only obtained with hepatic RNA from untreated animals (Fig. 3). These protected fragments were distinctly smaller than the fragment protected by the *in vitro* transcribed P450IIC6 (556–763), indicating that the probe hybridized to a hepatic RNA which was not completely complementary to it.

Discussion

The P450IA family consists of two members, termed P450IA1 and P450IA2. These two genes appear to code for two proteins that were named cytochrome P-450*c* and *d* (*10*). Early immunological data of Guengerich and Mason (*4*) showed that these proteins occur in the liver of untreated animals and are strongly induced by methylcholanthrene. In addition, these enzymes have been found in several extrahepatic tissues. However, cytochrome P450IA1 and IA2 were not analyzed differentially. In a recent study (*5*), the amount of cytochrome P450IA1 protein was determined selectively in several tissues. In contrast to our study (Fig. 1), P450IA1 was found to be expressed in control liver and kidney, though at a 100-fold lower level than in the liver of

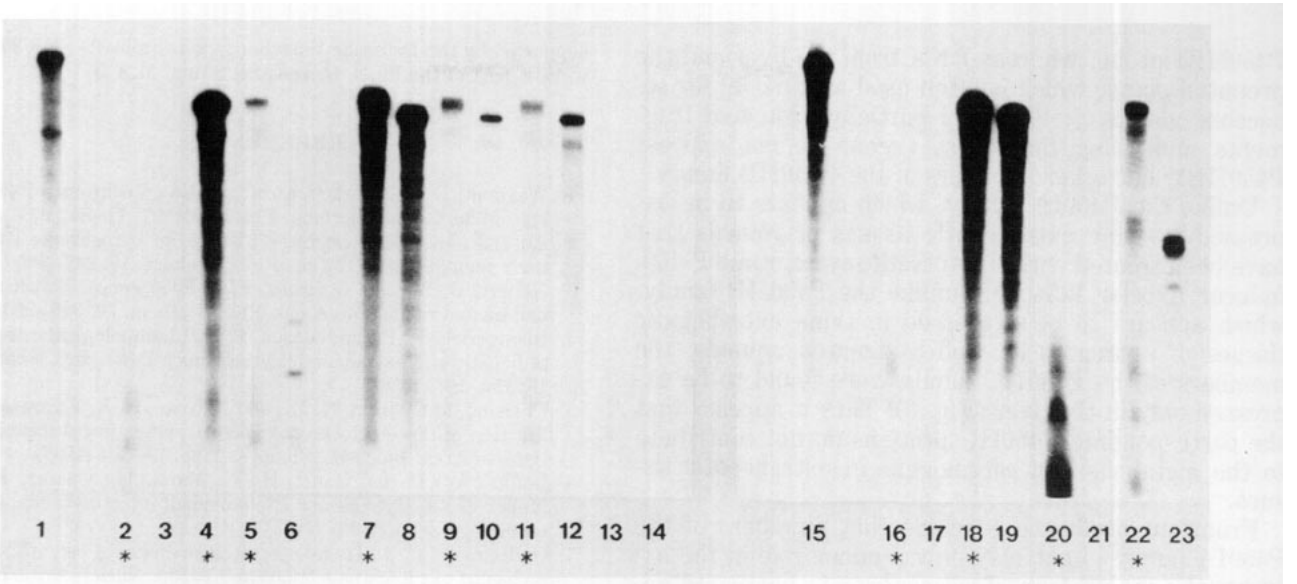

FIGURE 2. Detection of cytochrome P450IIB RNAs in various tissues. The P450IIB1 (133–499) anti-sense RNA probe was used in the nuclease protection assays of various RNA samples. An asterisk below the lane number indicates that the assay was performed with nuclease S1. Otherwise the assays were performed with RNAse A. The probe was incubated with the following RNA samples: (lanes 2 and 3) no RNA; (lane 4) P450IIB1 (133–499) sense transcript, (lanes 5 and 6) hepatic RNA from untreated animals; (lanes 7, 8, 18, and 19) hepatic RNA from Aroclor-treated animals; (lanes 9 and 10) lung RNA; (lanes 11 and 12) intestinal RNA; (lanes 13 and 14) testis RNA; (lanes 16 and 17) brain RNA; (lanes 20 and 21) kidney RNA; (lanes 22 and 23) preputial gland RNA. The poly(A$^+$) RNA from the lung, intestine, testis, brain, kidney and preputial gland was isolated from Aroclor-treated animals, whereas the lung RNA was from untreated animals; 1.5 μg of hepatic poly(A$^+$) RNA from treated animals or 5 μg of poly(A$^+$) RNA was used. The gel was subjected to autoradiography for 24 hr.

FIGURE 3. Detection of cytochrome P450IIC6 RNA in various tissues. The P450IIC6 transcript was used in the protection assay with RNA isolated from several tissues (lane 1) probe; (lane 2) liver; (lane 3) lung of treated animals; (lanes 4 and 5) kidney of untreated and of treated animals, respectively; (lanes 6 and 7) testes of untreated and treated animals, respectively; (lanes 8 and 9) intestinal mucosa of untreated and treated animals, respectively; (lane 10) adrenal gland; (lane 11) brain of male animals; (lane 12) brain of female animals; (lane 13) sense P450IIC6 transcript; (lane 14) anti-sense P450IIC6 transcript. In lanes 13 and 14 the indicated transcripts were incubated with the probe followed by RNAse A treatment. Treatment was with Aroclor 1254. Unless otherwise specified the tissues were from untreated animals.

methylcholanthrene-treated animals. By using a dilution series of mRNA from treated animals, we estimate that we should have detected a level of P450IA1 mRNA in the control liver and kidney, which is by a factor of 1000 lower than the level in the induced liver. Our data are supported by a recent study which found, using oligomer probes, that only P450IA2, but not P450IA1, is expressed in the liver of untreated animal (*11*).

In the study displayed in Figure 1, the signal consisted of a fully protected fragment, and no distinct partially protected fragments were obtained. However, by using a probe covering the 5′ end of the P450IA1 cDNA in the protection assay of RNA isolated from treated animals instead of the P450IA1 (1957–2620) probe, we detected shorter protected fragments in addition to the fully protected probe (data not shown). Only these partially protected fragments were found in the assay of mRNA isolated from untreated animals. We think that the partially protected fragments were derived from the P450IA2 RNA, indicating that only P450IA2 and not P450IA1 is expressed in the liver of untreated animals. Thus our data show, using the highly sensitive RNAse A protection assay, that besides the liver, which expresses P450IA2, no other organ of untreated animals expresses P450IA genes. This observation raises the question whether the corresponding proteins play a role in chemical carcinogenesis, which seems unlikely unless one assumes that at least in humans those proteins are expressed due to exposure to environmental compounds. On the other hand, our study showed (Fig. 2) that members of the P450IIB family, some of which encode the major phenobarbital-inducible cytochromes P-450, are expressed in the lung, the liver, and the preputial gland of untreated animals. However, for untreated animals, only RNA from the lung yielded a considerable amount of the fully protected

P450IIB1 probe, whereas RNA from the liver and the preputial gland, which is often used as a model for sebaceous glands, yielded only partially protected fragments, indicating that these organs do not express P450IIB1, but other members of the P450IIB family.

Unlike the P450IA family, which appears to be expressed in some extrahepatic tissues of animals that have been treated with the versatile cytochrome P-450-inducer Aroclor 1254, and unlike the P450IIB family, which appears to be expressed in some extrahepatic tissues of untreated as well as treated animals, the members of the P450IIC family were found to be expressed only in the liver (Fig. 3). Thus it appears that the corresponding P450IIC proteins do not contribute to the metabolism of carcinogens in extrahepatic tissues.

From our study we conclude that members of the P450IA family might play only a minor role in the extrahepatic metabolism of carcinogens in untreated animals but play an important role in the metabolism of xenobiotics after exposure of the animals to compounds such as Aroclor 1254. In contrast, some members of the P450IIB family seem to be expressed constitutively in some extrahepatic tissues such as the lung and may contribute significantly to the metabolism of xenobiotics in those tissues.

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